Supplementary Material (ESI) for Chemical Communications

Supporting information for

A microfluidic approach for high-throughput droplet interface bilayer (DIB) formation

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1) Experimental Details

Vesicle Preparation

Lipids used in these experiments were 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC, Avanti Polar Lipids) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, Avanti Polar Lipids). The corresponding vesicle solutions were prepared as follows. Correct volumes of the appropriate lipid were deposited from chloroform stocks into round bottom flasks to provide 2 mg of lipid. Chloroform was removed in the first instance under a stream of nitrogen and subsequently dried overnight in a desiccator to yield a thin lipid film. This was then re-hydrated in 1 ml of buffer, either non-fluorescent (10 mM HEPES, 200 mM KCl, pH 7.07) or fluorescent (250 µM fluorescein, 10 mM HEPES, 200 mM KCl, pH 7.60), to yield a final lipid concentration of 2 mg/ml. After vortexing to ensure complete dissolution, the samples were put through 5 freeze-thaw cycles and extruded 21 times through a 100 nm polycarbonate membrane (Avanti Polar Lipids). The extruded vesicle solutions were wrapped in foil and stored at 4 ^{0}C.

Device Fabrication

'Thick' polyethylene (PE) tubing (380 µm ID, Harvard Apparatus UK) was inserted into one end of a hollow square capillary (borosilicate glass, 1 mm x 1 mm x 50 mm, Composite Metal Services Ltd.), secured and sealed using Araldite (RS Components) and dried (60 °C, 1 h). 'Thin' PTFE (polytetrafluoroethylene) ultramicrobore tubing (0.004" ID, Cole-Parmer Instrument) was inserted into the opposite entrance of the capillary and sealed in an analogous manner. Initial experiments with vesicles comprised of DPhPC were performed with only one length of thin tubing inserted into the capillary. Subsequent experiments with DOPC vesicles were performed with four lengths of thin tubing inserted into the glass capillary, as this was found to equalise the flow conditions throughout the length of the channel.

Water-in-Oil Droplet Formation

Aqueous droplets (vesicle solutions described above) were created in hexadecane oil (99%, Sigma-Aldrich). A syringe pump (PHD 2000, Harvard Apparatus) working in refill mode was used to aspirate liquid *via* the thin PTFE tubing. A two channel autosampler was used to move the tip of the tubing into oil and vesicle solutions alternately, thereby sucking vesicle droplets into the tubing. Small droplets (Fig. 2B) were formed at low volumetric flow rates (3 µl/min), whilst larger droplets (Fig. 2C) were formed at high volumetric flow rates (6 µl/min) but at a lower frequency (when using DPhPC vesicle solutions). When using DOPC (for leakage experiments described below), higher flow rates were needed to create droplets of intermediate size. This is likely to be due to differences in the interfacial tension or viscosities of the aqueous phase. Droplets were then pumped into the glass channel described above.

Fluorescent Leakage Experiments

Leakage assays were performed on ABAB droplet patterns achieved through use of the two channel autosampler described above. Once droplets were in the glass channel, the syringe pump was stopped. Bright field images were taken at 20 ms exposures (5x magnification). Fluorescence images were taken using a fluorescein isothiocyanate (FITC) filter (480/40 excitation, 488 long pass, Chroma Technology Corp.) with a 2 s exposure (5x magnification). Two neutral density filters were used for the fluorescent images, to prevent photo-bleaching (25ND6 and 25ND25, Olympus). Illumination was through a mercury lamp (Olympus). Images were taken every 20 min up to 1 hour, then every 30 min up to 2 hours and finally at 3 hours and 20 hours after the start of the experiment (not all images are shown in Fig. 3). Bright field images show that no droplets merged and that experimental conditions remained the same throughout.

Control Experiments

Formation of water-in-oil droplets was unsuccessful when there was no lipid present in the buffer solution. This shows that lipid is required in order to form DIBs.

To test whether fluorescein leaks from the aqueous phase into the oil phase, fluorescent buffer (500 µl, prepared as described above) and hexadecane oil (500 µl) were placed in the same glass vial (where an aqueous-oil interface was formed) and left in a darkened environment for five days. Aliquots (300 µl) of each phase were pipette into a well-plate and fluorescence measurements carried out on a Cary Eclipse (Varian Inc.) fluorescence spectrophotometer with excitation at 490 nm and emission collected from 500 to 700 nm. Excitation and emission slits were both set to 5 nm, and a medium scan rate and photomultiplier tube detector voltage utilised (600 V). Controls were also conducted with fresh samples of hexadecane and fluorescent buffer for comparison. No discernable leakage of fluorescein into the oil phase was detected.

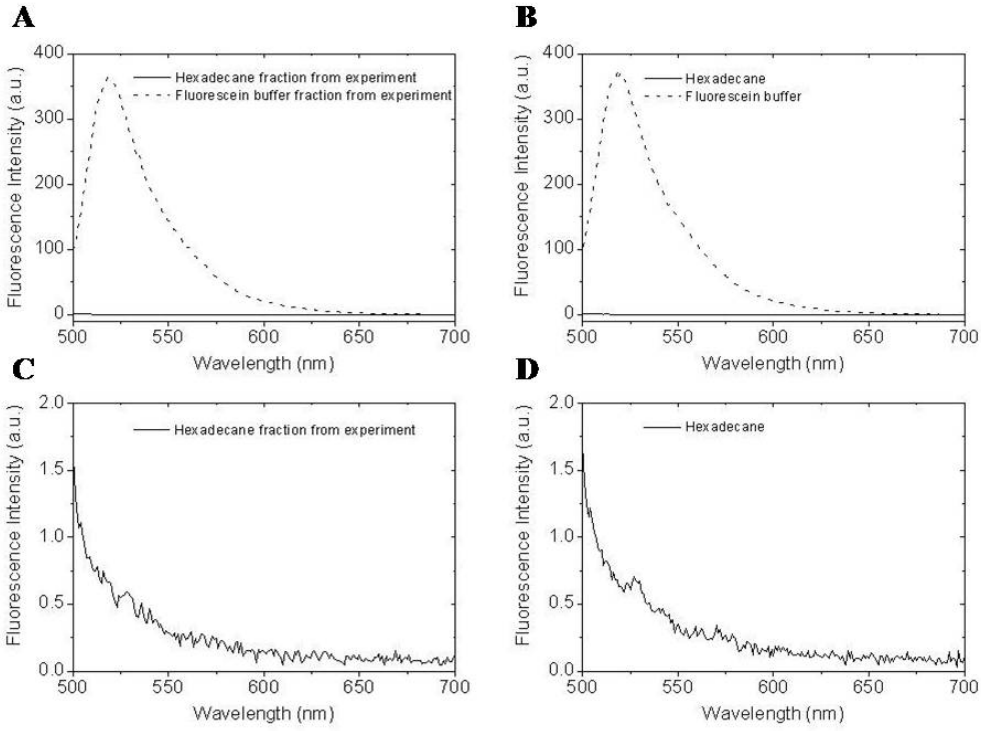

Fig. 1 Graphs showing the results of the fluorescence measurements for the control experiment. A) Fluorescence measurements of hexadecane oil (solid line) and buffer containing fluorescein (dashed line) obtained from samples kept in the same container over the course of five days, where C) is a close-up version. This demonstrates that there is no leakage of fluorescein into the oil. B) Fluorescence measurements of hexadecane oil (solid line) and buffer containing fluorescein (dashed line) obtained from fresh samples are included for comparison, where D) is a close-up version of B. This shows that there is no fluorescence present innately in hexadecane oil.

Imaging

All images were taken with an Olympus DP71 CCD camera mounted on an Olympus BX51 upright microscope. The software used for image acquisition was analySIS (Olympus Soft Imaging Solutions GmbH, Version 5.0). Fluorescent images were processed using IrfanView (freeware by Irfan Skiljan, 2009).

2) Movies

Movie 1 - 'Formation of Droplet Interface Bilayer'. This movie shows DIB formation between a non-fluorescent droplet (2 mg/ml DPhPC) and a fluorescent droplet (2 mg/ml DPhPC with 250 μM fluorescein).

Movie 2 – 'Formation of Large Droplets'. Movie showing the introduction of large droplets (2 mg/ml DPhPC) into the glass channel. The volumetric flow rate is 6 μl/min. The PTFE tubing where droplets are carried can be seen in the lower left hand corner, the interior diameter of which is 102 μm.

Movie 3 – 'Small Droplets Filling a Chamber'. Movie showing the introduction of small droplets (2 mg/ml DPhPC) into the glass channel. They stack in three dimensions and fill the chamber, forming DIBs. The volumetric flow rate is 3 μl/min. The PTFE tubing where droplets are carried can be seen in the lower left hand corner, the interior diameter of which is 102 μm.

Movie 4 – 'Formation of DIBs with DOPC'. Movie showing formation of a DIB between two DOPC (2 mg/ml) droplets. Droplets were formed with volumetric flow rates of 12 μl/min.